

## CLAIMS

What is claimed is:

1. An isolated antibody or a fragment thereof that specifically binds native FcγRIIB with greater affinity than said antibody or fragment thereof binds FcγRIIA.
2. The antibody of claim 1, wherein said binding agonizes at least one activity of FcγRIIB.
3. The antibody of claim 2, wherein said activity is inhibition of B cell receptor-mediated signaling.
4. The antibody of claim 2, wherein said activity is inhibition of FcεRI-induced mast cell activation.
5. The antibody of claim 2 which inhibits activation of B cells, B cell proliferation, antibody production, intracellular calcium influx, or activity of one or more downstream signaling molecules in the FcγRIIB signal transduction pathway.
6. The antibody of claim 5, wherein said downstream signaling molecule is PLCγ, MAPK, Btk, or Akt.
7. The antibody of claim 2 which enhances phosphorylation of FcγRIIB and/or recruitment of one or more downstream signaling molecules in the FcγRIIB signal transduction pathway.
8. The antibody of claim 7, wherein said downstream signaling molecule is SHIP.
9. The antibody of claim 1, wherein said binding antagonizes at least one activity of FcγRIIB.
10. The antibody of claim 9, wherein said activity is activation of B cell receptor-mediated signaling.
11. The antibody of claim 9, wherein said activity is activation of FcεRI-induced mast cell activation.

12. The antibody of claim 9 which enhances B cell activity, B cell proliferation, antibody production, intracellular calcium influx, or activity of one or more downstream signaling molecules in the FcγRIIB signal transduction pathway.

13. The antibody of claim 12, wherein said downstream signaling molecule is Btk kinase, PLCγ, MAPK, or Akt.

14. The antibody of claim 9 which reduces phosphorylation of FcγRIIB and/or recruitment of one or more downstream signaling molecules in the FcγRIIB signal transduction pathway.

15. The antibody of claim 14, wherein said downstream signaling molecule is SHIP.

16. The antibody of claim 1, wherein said antibody is a monoclonal antibody.

17. The antibody of claim 1, wherein said antibody is a humanized antibody.

18. The antibody of claim 1, wherein said antibody is a human antibody.

19. The antibody fragment of claim 1, wherein said fragment is a F(ab')<sub>2</sub> fragment.

20. The antibody fragment of claim 1, wherein said fragment is a F(ab) fragment.

21. The antibody of claim 1, wherein said antibody is a single chain antibody.

22. A bispecific antibody comprising a first heavy chain-light chain pair that specifically binds FcγRIIB with greater affinity than said heavy chain-light chain pair binds FcγRIIA, and a second heavy chain-light chain pair that specifically binds a tumor antigen.

23. An isolated antibody or a fragment thereof that specifically binds native FcγRIIB with at least 2 times greater affinity than said antibody or fragment thereof binds native FcγRIIA.

24. The antibody of claim 1, wherein said antibody is operably linked to a heterologous polypeptide.

25. The antibody of claim 1, wherein said heterologous polypeptide is an antibody that immunospecifically binds a cell surface receptor.

26. The antibody of claim 1, wherein said heterologous polypeptide is an antibody that immunospecifically binds a tumor antigen.

27. The antibody of claim 1, wherein said antibody is conjugated to a therapeutic agent.

28. The antibody of claim 27, wherein said therapeutic agent is a cytotoxin.

29. The antibody of claim 28, wherein said cytotoxin is paclitaxel, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, epirubicin, or cyclophosphamide.

30. The antibody of claim 1 which blocks binding of an Ig-Fc to FcγRIIB.

31. The antibody of claim 30 which enhances an immune response.

32. The antibody of claim 31, wherein said enhanced immune response is an increase in antibody dependent cellular response.

33. A method for producing a monoclonal antibody that specifically binds FcγRIIB with greater affinity than said monoclonal antibody binds FcγRIIA, said method comprising:

- (a) immunizing FcγRIIA transgenic mice with purified FcγRIIB or an immunogenic fragment thereof;
- (b) producing hybridoma cells lines from spleen cells of said mice;
- (c) screening said hybridoma cell lines for one or more hybridoma cell lines that produce antibodies that specifically bind FcγRIIB with greater affinity than the antibodies bind FcγRIIA.

34. An antibody produced by the method of claim 33.
35. The method of claim 33, wherein said immunogenic fragment is the soluble extracellular domain of FcγRIIB.
36. A mouse monoclonal antibody produced by clone 3H7, having ATCC accession number PTA-4592.
37. The antibody of claim 36, wherein said antibody has been humanized.
38. A mouse monoclonal antibody produced by clone 2B6, having ATCC accession number PTA-4591.
39. An isolated antibody or fragment thereof that competes for binding with the antibody of claim 36, and binds FcγRIIB with greater affinity than said antibody or fragment thereof binds FcγRIIA.
40. A hybridoma cell line 3H7, having ATCC accession number PTA-4592.
41. The antibody of claim 38, wherein said antibody has been humanized.
42. An isolated antibody or a fragment thereof that competes for binding with the antibody of claim 38, and binds FcγRIIB with greater affinity than said antibody or fragment thereof binds FcγRIIA
43. A hybridoma cell line 2B6, having ATCC accession number PTA-4591.
44. An isolated nucleic acid comprising a nucleotide sequence encoding a heavy chain or a light chain of the antibody or fragment thereof of claim 1.
45. A vector comprising the nucleic acid molecule of claim 44.
46. A vector comprising a first nucleic acid molecule encoding a heavy chain and a second nucleic acid molecule encoding a light chain, said heavy chain and light chain being of the antibody or fragment thereof of claim 1.
47. The vector of claim 45 which is an expression vector.

48. A host cell containing the vector of claim 45.
49. A host cell containing a first nucleic acid operably linked to a heterologous promoter and a second nucleic acid operably linked to the same or a different heterologous promoter, said first nucleic acid and second nucleic acid encoding a heavy chain and a light chain, respectively, of the antibody of claim 1.
50. A method for recombinantly producing a Fc $\gamma$ RIIB specific antibody, said method comprising: (i) culturing in a medium the host cell of claim 48, under conditions suitable for the expression of said antibody; and (ii) recovery of said antibody from said medium.
51. A method of treating cancer in a patient having a cancer characterized by a cancer antigen, said method comprising administering to said patient a therapeutically effective amount of a first antibody or fragment thereof that specifically binds Fc $\gamma$ RIIB with greater affinity than said antibody or fragment thereof binds Fc $\gamma$ RIIA, and a second antibody that specifically binds said cancer antigen and is cytotoxic.
52. The method of claim 51, wherein said cancer is breast, ovarian, prostate, cervical or pancreatic cancer.
53. The method of claim 51, wherein said cytotoxic antibody is Herceptin®, Rituxan®, IC14, PANOREX™, IMC-225, VITAXIN™, Campath 1H/LDP-03, LYMPHOCIDE™, or ZEVLIN™.
54. The method of claim 51, wherein said cancer antigen is MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, N-acetylglucosaminyltransferase, p15, beta-catenin, MUM-1, CDK4, HER-2/neu, human papillomavirus-E6, human papillomavirus-E7, or MUC-1.
55. The method of claim 51, wherein said cancer antigen is a breast, ovarian, prostate, cervical, or pancreatic carcinoma antigen.
56. The method of claim 51 further comprising the administration of one or more additional cancer therapies.

57. The method of claim 56, wherein said additional cancer therapy is selected from the group consisting of chemotherapy, immunotherapy, radiation therapy, hormonal therapy, or surgery.

58. The method of claim 51, wherein said patient is human.

59. The method of claim 58, wherein said first antibody is a human antibody or a humanized antibody.

60. A pharmaceutical composition comprising (i) a therapeutically effective amount of the antibody or fragment thereof that specifically binds FcγRIIB with greater affinity than said antibody or fragment thereof binds FcγRIIA; (ii) a cytotoxic antibody that specifically binds a cancer antigen; and (iii) a pharmaceutically acceptable carrier.

61. The pharmaceutical composition of claim 60, wherein said first antibody is a human or humanized antibody.

62. The pharmaceutical composition of claims 60 or 61, wherein said second antibody is a human or humanized antibody.

63. The pharmaceutical composition of claim 60 further comprising one or more additional anti-cancer agents.

64. The pharmaceutical composition of claim 63, wherein said anti-cancer agent is a chemotherapeutic agent, a radiation therapeutic agent, a hormonal therapeutic agent, or an immunotherapeutic agent.

65. A method of treating an autoimmune disorder in a patient in need thereof, said method comprising administering to said patient a therapeutically effective amount of the antibody or fragment thereof of claim 1.

66. The method of claim 65, wherein said autoimmune disorder is rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Rieter's Syndrome, psoriasis, or lupus erythematosus.

67. The method of claim 65 further comprising administering to said patient a therapeutically effective amount one or more anti-inflammatory agents.

68. The method of claim 65 further comprising administering to said patient a therapeutically effective amount one or more immunomodulatory agents.

69. The method of claim 68, wherein at least one immunomodulatory agent is a small organic molecule.

70. The method of claim 69, wherein the small organic molecule is methotrexate, leflunomide, cyclophosphamide, cyclosporin A, FK506, mycophenolate mofetil, rapamycin, mizoribine, deoxyspergualin, brequinar, malonitrolamide, steroid, or corticosteroid.

71. The method of claim 67, wherein at least one anti-inflammatory agent is a non-steroidal anti-inflammatory drug.

72. The method of claim 71, wherein the non-steroidal anti-inflammatory drug is aspirin, ibuprofen, diclofenac, nabumetone, naproxen, or ketoprofen.

73. A method for treating or preventing an IgE-mediated allergic disorder in a patient in need thereof, comprising administering to said patient a therapeutically effective amount of the antibody or fragment thereof of claim 2.

74. The method of claim 73, wherein said IgE-mediated allergic disorder is asthma, allergic rhinitis, gastrointestinal allergies, eosinophilia, conjunctivitis, or glomerular nephritis.

75. The method of claims 65 or 73, wherein said patient is human.

76. The method of claim 75, wherein said antibody is a humanized antibody or a human antibody.

77. A method of enhancing an antibody mediated cytotoxic effect in a subject being treated with a cytotoxic antibody, said method comprising administering to said patient the antibody or fragment thereof of claim 1 in an amount sufficient to enhance the cytotoxic effect of said cytotoxic antibody.

78. A method of diagnosis of an autoimmune disease in a subject comprising:  
(a) contacting a biological sample from said subject with an effective amount of the antibody or a fragment thereof of claim 1; and  
(b) detecting binding of said antibody or fragment thereof,  
wherein detection of said detectable marker above a background or standard level indicates that said subject has an autoimmune disease.

79. The method of claim 78, wherein said detectable marker is a chemiluminescent, enzymatic, fluorescent, or radioactive label.

80. A method of enhancing an immune response to a vaccine composition in a subject, said method comprising administering to said subject an antibody or fragment thereof that specifically binds Fc $\gamma$ RIIB with greater affinity than said antibody or fragment thereof binds Fc $\gamma$ RIIA, and a vaccine composition, said antibody or fragment thereof being administered in an amount effective to enhance the immune response to said vaccine composition in said subject.

81. An isolated antibody or a fragment thereof that specifically binds Fc $\gamma$ RIIB with 4 times greater affinity than said antibody or fragment thereof binds Fc $\gamma$ RIIA.

82. An isolated antibody or a fragment thereof that specifically binds Fc $\gamma$ RIIB with 6 times greater affinity than said antibody or fragment thereof binds Fc $\gamma$ RIIA.

83. An isolated antibody or a fragment thereof that specifically binds Fc $\gamma$ RIIB with 8 times greater affinity than said antibody or fragment thereof binds Fc $\gamma$ RIIA.

84. An isolated antibody or a fragment thereof that specifically binds Fc $\gamma$ RIIB with 10 times greater affinity than said antibody or fragment thereof binds Fc $\gamma$ RIIA.

85. An isolated antibody or a fragment thereof that specifically binds Fc $\gamma$ RIIB with 100 times greater affinity than said antibody or fragment thereof binds Fc $\gamma$ RIIA.

86. An isolated antibody or a fragment thereof that specifically binds Fc $\gamma$ RIIB with 1000 times greater affinity than said antibody or fragment thereof binds Fc $\gamma$ RIIA.



87. An isolated antibody or a fragment thereof that specifically binds FcγRIIB with  $10^4$  times greater affinity than said antibody or fragment thereof binds FcγRIIA.

88. An isolated antibody or a fragment thereof that specifically binds FcγRIIB with  $10^5$  times greater affinity than said antibody or fragment thereof binds FcγRIIA.

89. An isolated antibody or a fragment thereof that specifically binds FcγRIIB with  $10^6$  times greater affinity than said antibody or fragment thereof binds FcγRIIA.

90. A pharmaceutical composition comprising (i) a therapeutically effective amount of the antibody or fragment thereof that specifically binds FcγRIIB with greater affinity than said antibody or fragment thereof binds FcγRIIA; and (ii) a pharmaceutically acceptable carrier.

91. A method for producing a monoclonal antibody that specifically binds FcγRIIB with greater affinity than said monoclonal antibody binds FcγRIIA, said method comprising:

- (a) immunizing FcγRIIA transgenic mice with purified FcγRIIB or an immunogenic fragment thereof;
- (b) booster immunizing said mice for a time sufficient to elicit an immune response;
- (c) producing hybridoma cells lines from spleen cells of said mice;
- (d) screening said hybridoma cell lines for one or more hybridoma cell lines that produce antibodies that specifically bind FcγRIIB with greater affinity than the antibodies bind FcγRIIA.

92. The method of claim 91, wherein said mice are booster immunized at least four times over a four month period.

93. A method of treating cancer in a patient, said method comprising administering to said patient a therapeutically effective amount of an antibody or a fragment thereof that specifically binds FcγRIIB with greater affinity than said antibody or fragment thereof binds FcγRIIA.

94. A method of treating a B cell malignancy in a patient, said method comprising administering to said patient a therapeutically effective amount of an antibody

or a fragment thereof that specifically binds FcγRIIB with greater affinity than said antibody or fragment thereof binds FcγRIIA.

95. The method of claim 94, wherein said B cell malignancy is non-Hodgkin's lymphoma.

96. A method of treating a disease in a patient comprising administering a therapeutically effective amount of a first antibody or fragment thereof that specifically binds FcγRIIB with a greater affinity than said antibody or fragment thereof binds FcγRIIA, and a second antibody, wherein said second antibody does not mediate its therapeutic effect by cell killing.

97. The method of claim 96, wherein said second antibody is an anti-Fas antibody.

98. A method of treating a solid tumor in a patient having a tumor characterized by infiltration of a population of macrophages at the site of the tumor, said method comprising administering a therapeutically effective amount of a first antibody or fragment thereof that specifically binds FcγRIIB with a greater affinity than said antibody or fragment thereof binds FcγRIIA, wherein said antibody reduces the population of macrophages.

99. The method of claim 98 wherein said antibody reduces the population of macrophages by at least 80%.

100. A method of treating cancer in a patient having a cancer characterized by a cancer antigen, said method comprising administering to said patient a therapeutically effective amount of a first antibody or fragment thereof that specifically binds FcγRIIB with a greater affinity than said antibody or fragment thereof binds FcγRIIA, and a second antibody that does not bind said cancer antigen.

101. The method of claim 100, wherein said second antibody binds a cancer antigen expressed on a cell surrounding a tumor cell.

102. The method of claim 101, wherein said cell is a fibroblast cell or a stromal cell.

103. The method of claim 102, wherein said cancer antigen is fibroblast activation protein.

104. The antibody of claim 1, further comprising at least one modification in the Fc region.

105. The antibody of claim 104, wherein said antibody has an altered affinity for an FcγR.

106. The antibody of claim 104, wherein said antibody binds FcγRIIIA with a higher affinity than a comparable antibody comprising a wild-type Fc region binds FcγRIIIA.

107. The antibody of claim 104, wherein said antibody has an enhanced antibody mediated effector function relative to a comparable antibody comprising a wild-type Fc region.